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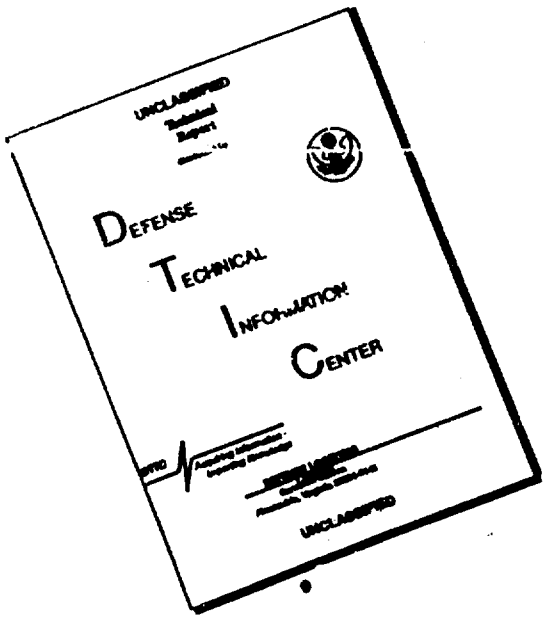

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Voprosy Pitaniya, 1961, vol. 20, [illegible]

Use of the Complement Binding Reaction [illegible]

Determination of Botulinal [illegible]

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Presently, the biological test is the only widely approved method of determination of botulinal toxins. Nevertheless, this test has a number of disadvantages, because the correlations of the toxin and antitoxin are determined indirectly by experiments on animals. The individual sensitivity of animals must always be considered with the conduct and evaluation of experiments on animals; this reflects upon the exactness of the results, particularly when we deal with small doses of toxin like 10 to 5 to 1 Dlm. This manifests itself quite clearly in neutralization experiments with small doses of toxin by a specific or nonspecific serum. Furthermore, neither at all laboratories, nor at all times, are experimental animals available in a weight that is essential to arrangement of a biological test, and this also has a very important effect on practical research. Consequently, we

were compelled to develop other methods of determination of botulinal toxin. Thus, we developed: a color reaction for determination of the toxin (G.V.KOLOBOLOTSKII, 1951); the method of determination of phagocytic index (S.M.MINERVIN, S.P.ZHAN and M.I.CHERVYAKOVA, 1950); the ring precipitation reaction (M.YA. MINDER and P.A. CHERTKOVA, 1940); the complement fixation reaction (V.F.NIKOLENKO and A.A. BURNOS, 1937; A.A. BURNOS and S.A. FRIDMAN, 1957; GUNNISON and SCHOENHOLZ, 1927; STEWART, 1932). A considerable amount of work was devoted to the application of serological reaction in determination of botulinal toxin and this has its reason. The authors were trying to determine the toxin by a direct method with the aid of antibodies. The serological reaction is a more objective method of a quantitative determination of antigens, because it offers more exact and constant indices of the contents of antigens, or antibodies and it differentiates more finely the variations of small doses of toxin. Thus, we paused at the most sensitive serological reaction, - the complement fixation reaction (CFR), in order to determine botulinal toxin.

Inasmuch as the systematic part of the reaction was not fully discussed in the afore-mentioned reports, it is self-evident that a problem emerged to define more accurately the arrangement method of the CFR reaction for a quantitative determination of the toxin and to compare its sensitivity with that of the biological test. The authors adopted the universal method of the CFR determination using a 2.5 ml volume. They used as antigen a filtered broth culture of Cl. botulinum in dilution of 1:4 (STEWART), or undiluted in a

volume of 0.1 to 0.4 ml (A.A.BURNOS and S.N. [illegible]). [illegible] used as antigen either the therapeutic [illegible] in a dilution of 1:10 and 1:50 (A.A.BURNOS and S.[illegible], [illegible]), or [illegible] a rabbit in dilution of 1:50 (BURNISON and SCHLEMMER). The [illegible] (within 1 hour) was carried out at 37°C.

We set the CFR according to the universally accepted method in a volume of 2.5 ml and we used, at the same time, the following components: for antigen - dry botulinal toxin type A of the series No.14 and 18 (obtained from N.F.GAMALEI'S Institute of Epidemiology and Microbiology); the toxin was diluted with a physiological solution and every batch of toxin was titrated on white mice. The toxin of the No.14 series contained in 1 mg 30,000 Dlm., while that of the No.18 series - 10,000 Dlm (T.N.: or MLD). We used for antibodies either the usual therapeutic antibotulinal serum, obtained from I.I.MECHNIKOV'S Scientific Research Institute for Vaccines and Serums, Moscow, or the type-specific rabbit's serum. We obtained the latter from rabbits immunized with adsorbent anatoxin type A. The rabbits' antiserums contained from 32 to 126 BU in 1 ml. We used in reaction a dry complement of the series No.189 obtained from I.I.MECHNIKOV'S Institute. Conforming with particulars, we carried out the fixation: 1) at 37°C in the course of 1 hour, and 2) at 2°C inthe course of 18 hours. The results were studied in subsequent days after we removed experimental test tubes from thermostatic control and exposed them to room temperature for 2 hours. Then, the tubes were placed in refrigeration for the night. With suppression of hemolysis, we took into account the reactions

at +++ and ++++. We obtained negative results from a preliminary test in CFR using four series of antibotulinal horse serum type A in a 1:50 dilution (series No.14 and 18) of the type A toxin, of which the active dose comprised 100 Dlm in 1 ml. The research of [illegible] and PARKER (1923), PITTMAN and GOODNER (1935), GOEBEL and [illegible] (1936), as well as our research with botulinal anatoxin proved that the complement fixation does not occur when antitoxic horse serum is used in reaction. We tested 3 series of rabbits' antiserums in a dilution of 1:50 in CFR with the same two series (No.14 and 18) of the type A toxin in identical doses. The obtained results were negative. In our work we demonstrated with the aid of the CFR pertinently to determination of antigen activity of botulinal anatoxin that the botulinal antigen-antiserum complex fixes but a small amount of complement, which is not detected with the CFR results in accordance with the universally adopted method. Yet, it was possible to determine by fixation small amounts of the complement, thus the sensitivity of the CFR has increased with the use in reaction, of hemolytic system that contained 0.1% of erythrocytes. First, we retitrated the hemolytic unit to small amounts of the complement. Prior to the arrangement of the basic experiment with the CFR, we determined the hemolytic unit of the complement, the active dose of the toxin and that of the immune serum. For this reason we carried out the complement titration per se and the complement titration in the presence of ascending dilutions of antigen and serum. We took into account as a complement unit the highest dilution of the complement that afforded a

full hemolysis. The active dose of antigen comprised one half of its quantity with which the hemolytic unit of the complement remained unchanged. In this way we selected the active dose of the serum. One hemolytic unit of the complement comprised 0.1 ml in a 1:30 dilution. The active dose of the toxin comprised 100 Dlm. We used in reaction the doses in the range of 50 to 25 to 10 to 5 and 1 Dlm in a volume of 0.5 ml. The active dose of immune serum comprised 0.5 ml in a 1:100 dilution. We used in the experiment 1½ (0.15 ml) and 2 (0.2 ml) hemolytic units of the complement in a 1:30 dilution. As a control measure we used in every experiment double doses of antigen, and serum with 1½ and 2 units of the complement. All controls of antigen and serum yielded a full hemolysis in all our experiments.

We submit in Table 1 the results of the experiments involving the set-up of the CFR with two series of toxin. The fixation was accomplished at 2°C temperature within 18 hours. Negative results were obtained from the fixation at 37°C temperature within 1 hour.

It is obvious from Table 1 that, with the aid of the CFR, one can determine quantitatively botulinal toxin type A. In two series of toxins studied by us we determined the toxin in doses of 5 to 10 Dlm. As to the sensitivity, the serological method of determination of botulinal toxin is somewhat inferior to the biological test.

Conclusions

1. The CFR in our modification can be used for a quantitative

Table 1

Determination of Botulin Toxin with the CFR Method

Dilution of immune serum	Dose of the complement	Dose of toxin in Dlm									
		Series No. 14				Series No. 16					
		50	25	10	5	1	50	25	10	5	1
1:50	1½ units	++++	+++	+++	++	+	++++	+++	+++	+++	++
1:50	2 units	++++	+++	++	++	+	++++	+++	++	++	+

determination of botulinal toxin type A in aqueous medium.

2. With the aid of the CFR, one can accomplish a determination of botulinal toxin type A in doses of 5 to 10 Dlm (MLD).

Literature Cited

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Summary (copied)

The present work dealt with the use of the complement fixation test in one of its modification for a qualitative ~~(clot)~~ determination of the A type botulin toxin in an aqueous medium. This reaction permitted to estimate the above toxin in doses of 5 to 10 Dlm. By its accuracy the described procedure is somewhat inferior to the biological test.